



Detection of TR₃₄/L98H CYP51A Mutation through Passive Surveillance for Azole-Resistant *Aspergillus fumigatus* in the United States from 2015 to 2017

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ABSTRACT The emergence of azole-resistant *Aspergillus fumigatus* has become a clinical problem in many parts of the world. Several amino acid mutations in the azole target protein Cyp51Ap contribute to this resistance, with the most concerning being the environmentally derived TR₃₄/L98H and TR₄₆/Y121F/T289A mutations. Here, we performed passive surveillance to assess a sample of the *A. fumigatus* population in the United States for the presence of these mutations. We found 1.4% of those isolates to exhibit elevated MIC via broth microdilution, and five of those isolates harbored the TR₃₄/L98H mutation.

KEYWORDS *Aspergillus fumigatus*, TR₃₄/L98H, TR₄₆/Y121F/T289A, azole resistance, CYP51A mutation

The primary etiologic agent of aspergillosis in the United States is *Aspergillus fumigatus*. While voriconazole is currently recommended by the Infectious Disease Society of America as the primary therapy for aspergillosis, the detection of azole resistance among isolates of *A. fumigatus* has been reported in many parts of the world (1–10). The most common mechanism of resistance is a mutation in CYP51A, the gene encoding the drug target protein. Numerous mutations with clinical relevance have been identified, with the most frequent being TR₃₄/L98H and TR₄₆/Y121F/T289A (11). These two mutations are of particular concern, as evidence suggests that they are environmentally derived through the extensive use of agricultural fungicides and occur in isolates from azole-naïve patients (12, 13).

Previously, we evaluated 1,026 *A. fumigatus* isolates, collected from across the United States between 2011 and 2013, for resistance to azoles and found no isolates with either the TR₃₄ or TR₄₆ mutation (14). Since that time, isolates with TR₃₄ mutation were reported from patients in Pennsylvania in 2010 and 2012, and isolates with TR₄₆ were reported from patients in Arizona in 2008 and Michigan in 2014, indicating that isolates with this mechanism of drug resistance are present in the United States (9, 15). Routine susceptibility testing of molds is uncommon in U.S. hospital laboratories, so the extent of azole resistance of *A. fumigatus* in this country is unknown. Here, we report on current U.S. surveillance for azole-resistant *A. fumigatus* and an evaluation of CYP51A mutations in resistant isolates.

Calls for the submission of *A. fumigatus* isolates were issued through the Centers for Disease Control and Prevention website (<https://www.cdc.gov/fungal/pdf/a-fumigatus-isolate-surveillance.pdf>) as well as the American Society for Microbiology (ASM) listservs DivC and ClinMicroNet. A total of 1,356 isolates of *A. fumigatus* were collected from September 2015 to April 2017. Species were confirmed as described previously (14).

Thirty-one hospitals, clinics, and state public health laboratories contributed isolates from 35 states and Washington, DC (Fig. 1). Many isolates were submitted from states/areas which were not previously involved in the earlier surveillance, including

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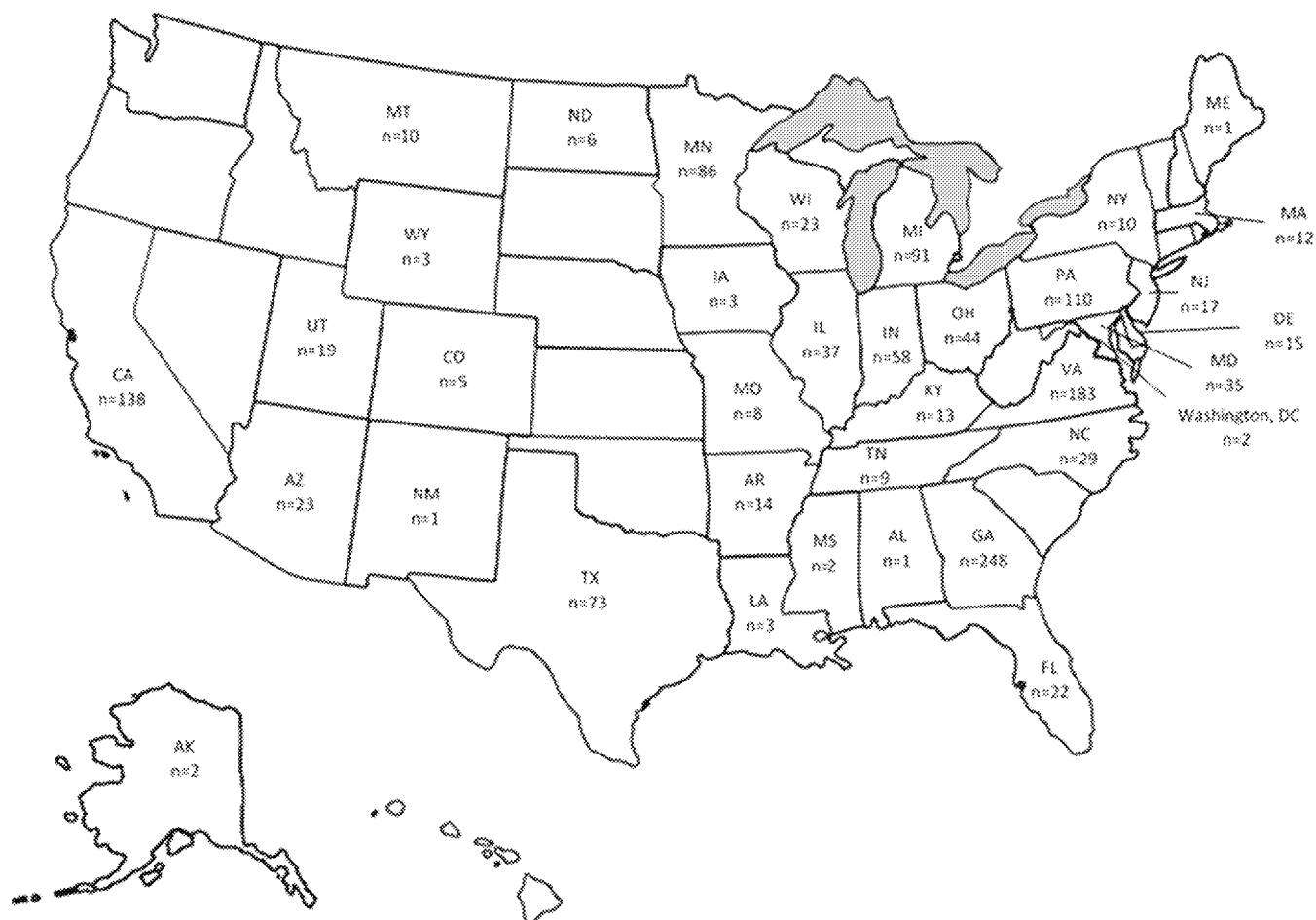


FIG 1 States of origin of 1,356 *A. fumigatus* isolates collected through passive surveillance, 2015 to 2017.

New Jersey ($n = 17$), Delaware ($n = 15$), Pennsylvania ($n = 110$), Maryland ($n = 35$), Washington, DC ($n = 2$), Ohio ($n = 44$), Kentucky ($n = 13$), Alabama ($n = 1$), Mississippi ($n = 2$), Wisconsin ($n = 23$), Arkansas ($n = 14$), Louisiana ($n = 3$), North Dakota ($n = 6$), Colorado ($n = 5$), New Mexico ($n = 1$), Utah ($n = 19$), and Alaska ($n = 2$) (14). Most isolates were from respiratory cultures (69%), but additional sources included wounds, tissues (e.g., eyes, ears, nails, and brain), body fluids, spinal specimens, and unknown.

Isolates were initially screened for elevated MICs to itraconazole and voriconazole using Etest strips, according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France), with minor modifications as previously described (14). Those isolates with elevated MICs to one or more of the antifungals tested via Etest ($\geq 2 \mu\text{g/ml}$ for itraconazole and voriconazole) were evaluated using broth microdilution according to CLSI guidelines (16) using custom-made frozen RPMI microbroth panels without indicator dye (Trek Diagnostics, Thermo Fisher Scientific, Oakwood Village, OH). An azole-resistant (TR_{34} mutant) isolate and an azole-susceptible isolate were used as controls in all testing, and the MIC values for these isolates remained within a tight range of 1 to 2 dilutions over the course of the testing (2). Although no interpretive breakpoints exist for *Aspergillus* species and the azoles, epidemiological cutoff values (ECVs) have been proposed, and these ECVs were used in susceptibility testing interpretation (17). The MICs that indicate a non-wild-type profile for itraconazole and voriconazole were $\geq 2 \mu\text{g/ml}$.

The vast majority (96.8%) of isolates were susceptible to both azoles tested. The MICs for Etests ranged from 0.032 to $6 \mu\text{g/ml}$ for voriconazole and 0.064 to $>32 \mu\text{g/ml}$ for itraconazole (Fig. 2). The MIC_{50} , MIC_{90} , and modal MIC for all isolates tested with

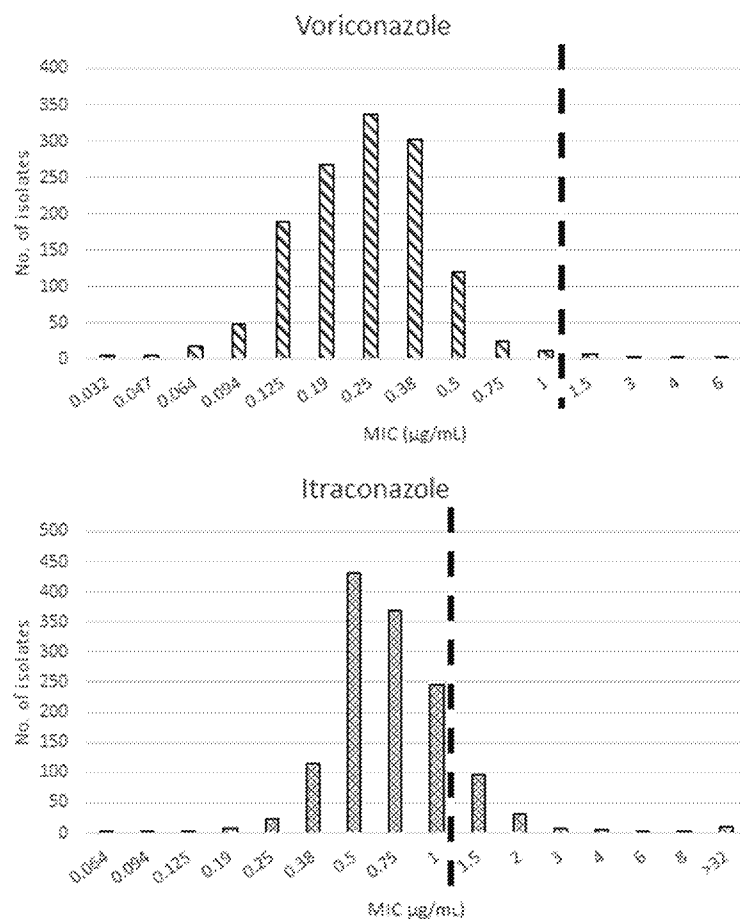


FIG 2 Susceptibility profiles for *A. fumigatus* isolates via Etest method. Dashed line represents the CLSI microdilution method ECV cutoff between wild type and non-wild type.

Etest were 0.25 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$, and 0.25 $\mu\text{g/ml}$, respectively, for voriconazole and 0.75 $\mu\text{g/ml}$, 1.5 $\mu\text{g/ml}$, and 0.5 $\mu\text{g/ml}$, respectively, for itraconazole. Of the 1,356 isolates, 43 isolates exhibited elevated MICs when screened with Etest and were further tested using broth microdilution. Of these 43 isolates, 20 exhibited elevated MICs to one or both antifungals via broth microdilution.

DNA sequence analysis of the CYP51A gene was performed for any isolate with elevated MICs confirmed via broth microdilution, as previously described (18). Sequencing revealed that 14 of 20 isolates contained an amino acid substitution in Cyp51A (Table 1). There were no novel mutations observed in these isolates; all have been previously described (14, 19). The most common substitution was I242V ($n = 6$), which was also the most common substitution found in previous surveillance (14). These isolates showed an MIC to voriconazole ranging from 0.06 to 2 $\mu\text{g/ml}$ and an MIC to itraconazole ranging from 2 to >16 $\mu\text{g/ml}$, as measured by broth microdilution (Table 1). Five isolates contained the TR₃₄/L98H mutation, either alone or in combination with additional mutations. Two of these isolates originated from Pennsylvania, two from Virginia, and one from California. These isolates had MICs to voriconazole ranging from 0.25 to 2 $\mu\text{g/ml}$ and MICs to itraconazole ranging from 4 to >16 $\mu\text{g/ml}$, as measured by broth microdilution. The TR₄₆ mutation was not identified in this collection of isolates.

A limitation of this monitoring system is the voluntary submission of isolates resulting in nonuniform geographic distribution of submitting labs. In the current surveillance, few to no isolates from much of the Midwest and the Pacific Northwest regions of the country were received. We also received very few isolates from the

TABLE 1 MICs of *A. fumigatus* isolates and amino acid substitutions in Cyp51A

Isolate no.	State of origin ^a	Yr received	Amino acid substitution(s) in Cyp51A	MIC ($\mu\text{g/ml}$) to ^b :	
				Itraconazole	Voriconazole
1415	Georgia	2015	I242V	2	1
1417	Georgia	2015		2	0.5
1484	Indiana	2015		2	0.5
1549	Georgia	2015		2	0.5
1554	Georgia	2015		2	1
1709	Maryland	2015	P216L	2	0.06
1878	Indiana	2016	F46Y/M172V/N248T/D255E/E427K	2	0.5
1926	California	2016	F46Y/M172V/N248T/D255E/E427K	2	0.5
1990	Georgia	2016		2	1
2001	Pennsylvania	2016	TR ₃₄ /L98H	4	2
2105	Georgia	2016	I242V	2	1
2211	Georgia	2016	I242V	2	0.5
2241	California	2016	I242V	2	0.5
2242	California	2016		2	1
2254	Virginia	2016	TR ₃₄ /L98H/S297T/F495I	>16	1
2288	Georgia	2016	I242V	2	1
2305	Virginia	2016	I242V	2	1
2714	Pennsylvania	2017	TR ₃₄ /L98H	16	2
2768	Virginia	2017	TR ₃₄ /L98H	16	0.5
2889	California	2017	TR ₃₄ /L98H/S297T/F495I	8	0.25

^aThe state of origin is the home state of the patient and not necessarily the state of the laboratory which submitted the isolate.

^bMIC as measured by broth microdilution.

southeastern United States outside Georgia (Fig. 1). Therefore, these surveillance data do not capture an accurate geographic picture of *A. fumigatus* isolates carrying the TR₃₄ mutation, especially in underrepresented regions of the country or those with no submitted isolates. The number of resistant isolates reported here is likely a substantial underestimate of their true presence in the United States.

Environmentally acquired resistance to azole antifungals is not associated with a fitness cost to the organism (20). As a result, resistant isolates likely persist alongside wild-type isolates in the environment (the frequency with which this happens in the United States is unknown). We recently identified azole-resistant *A. fumigatus* containing the TR₃₄ mutation in an experimental peanut field in Georgia that had been treated with azole fungicides (18). In the present study, Georgia contributed more clinical isolates to our survey than any other state, but we did not observe the TR₃₄ mutation from any of these isolates. To our knowledge, no studies have been performed in Pennsylvania, Virginia, or California to determine if TR₃₄ or TR₄₆ could be identified in the environment.

In this report, 14 of 19 total confirmed azole-resistant isolates contained a CYP51A polymorphism (74%), although not all of these polymorphisms have been directly linked to azole resistance. However, there were additional isolates that exhibited elevated MICs via broth microdilution and did not contain such mutations (Table 1). This finding supports the idea that other resistance pathways exist for this organism and could be operative among these isolates (21). Additional molecular testing of these isolates is warranted.

This study was determined to be surveillance and not involving human subjects under 45 CFR 46.102(f) (22), and no institutional review board (IRB) review was required.

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